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PHENYLPROPANOID GLYCOSIDES FROM *CALCEOLARIA HYPERICINA**

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Key Word Index—*Calceolaria hypericina*; Scrophulariaceae; phenylpropanoid glycosides; calceolarioside C and D, verbascoside.

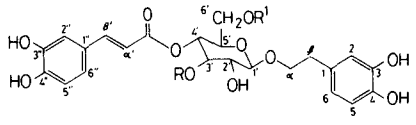
Abstract—Besides the known compound verbascoside, two new phenylpropanoid glycosides, calceolarioside C, 1′-*O*-β-D-(3,4-dihydroxy-β-phenyl)-ethyl-4′-*O*-caffeoyl-β-D-xylopyranosyl-(1‴ → 6′)-glucopyranoside, and calceolarioside D, 1′-*O*-β-D-(1-hydroxy-4-oxo-2,5-cyclohexadien)-ethyl-6′-*O*-caffeoylglucopyranoside, were isolated from the aerial parts of *Calceolaria hypericina*. The structures of the new compounds were elucidated by spectroscopic methods.

Several species of *Calceolaria* (Scrophulariaceae) are used in popular Chilean medicine as a tonic, stomachic and cicatrizing agents and against bacterial infections [2, 3]. A previous paper described the isolation of two new phenylpropanoid glucosides, calceolarioside A (**1**) and calceolarioside B(**2**), from the methanolic extract of *C. hypericina*, besides halleridone (**3**) [1]. Further purification by counter-current distribution (CCD) of the same extract resulted in the isolation of three additional minor compounds. One of them was readily identified as verbascoside (acteoside) (**4**) [4] whereas the other two were new phenylpropanoid glycosides, **5** and **6**, named calceolarioside C and D, respectively.

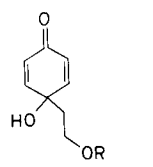
IR and UV spectra of calceolarioside C(**5**), mp 123–125°, $C_{28}H_{34}O_{15}$, were very similar to those of calceolarioside A and B [1]. Also ^1H NMR and ^{13}C NMR data (Tables 1 and 2, respectively) were closely related to those of **1**, except for the presence of additional peaks attributable to a sugar unit. The deshielding of H-6′$_a$ and H-6′$_b$ ($\Delta\delta$ = 0.2 ppm) allowed assignment of the linkage of the additional unit at position 6′ of the glucose, as was also confirmed by the corresponding shift of the C-6′ signal in the ^{13}C NMR spectrum ($\Delta\delta$ = +5 ppm), due to the *O*-alkyl substitution.

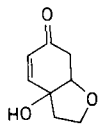
Total acid hydrolysis of **5** yielded D-glucose and D-xylose. ^1H NMR data confirmed the identification of the additional sugar unit as xylose, linked in the β-form [H-1‴ at δ4.25, doublet with J = 7.5 Hz and C(1‴) at δ105.2 [5], and thus the structure of 1′-*O*-β-D-(3,4-dihydroxy-β-phenyl)-ethyl-4′-*O*-caffeoyl-β-D-xylopyranosyl-(1‴→6′)-glucopyranoside was assigned to calceolarioside C.

Compound **5** therefore is an isomer of conandrioside, isolated from *Conandron ramoidioides* [6], in which the xylose linkage was assigned to position 3′. A diagnostic difference between the two compounds is represented by the chemical shift value of the anomeric proton of the xylose (δ4.52 in conandrioside). As a confirmation in the couple verbascoside (rhamnose in 3′) [4]/forsythoside A



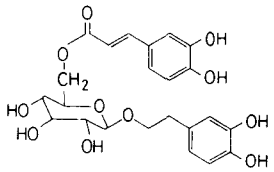
1 R = R^1 = H
4 R = rhamnose R^1 = H
5 R = H R^1 =





7 R = glucose
8 R = Ac

3



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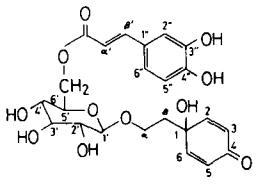

6

*Part 2 in the series 'Studies in *Calceolaria* genus'. For Part 1 see ref. [1].

Table 1. ^{1}H NMR spectral data of compounds **5** and **6***

H	5	6
2	6.68 (*d*, *J* = 2.5)	6.97 (*d*, *J* = 10.0)
3	—	6.09 (*d*, *J* = 10.0)
5	6.65 (*d*, *J* = 8.0)	6.09 (*d*, *J* = 10.0)
6	6.53 (*dd*, *J* = 2.5 and 8.0)	6.97 (*d*, *J* = 10.0)
α_1	4.03 (*m*)	3.94 (*m*)
α_2	3.72 (*m*)	3.66 (*m*)
2β	2.80 (*t*, *J* = 7.5)	2.05 (*m*)
1′	4.36 (*d*, *J* = 8.0)	4.28 (*d*. *J* = 8.0)
2′	3.60 (*m*)	3.20 (*dd*, *J* = 8.0 and 9.0)
3′	3.62 (*t*, *J* = 9.0)	3.33–3.58
4′	4.90 (*t*, *J* = 9.0)	3.33–3.58
5′	3.72 (*m*)	3.33–3.58
6′$_a$	3.86 (*dd*, *J* = 2.0 and 11.5)	4.33 (*dd*, *J* = 2.0 and 11.5)
6′$_b$	3.82 (*dd*, *J* = 5.0 and 11.5)	4.49 (*dd*, *J* = 5.0 and 11.5)
2″	7.02 (*d*, *J* = 2.5)	7.08 (*d*, *J* = 2.5)
5″	6.78 (*d*, *J* = 8.0)	6.80 (*d*, *J* = 8.0)
6″	6.92 (*dd*, *J* = 2.5 and 8.0)	6.95 (*dd*, *J* = 2.0 and 8.0)
α'	7.56 (*d*, *J* = 15.5)	7.59 (*d*, *J* = 15.5)
β'	6.26 (*d*, *J* = 15.5)	6.30 (*d*, *J* = 15.5)
1‴	4.25 (*d*, *J* = 7.5)	
2‴	3.15 (*dd*, *J* = 7.5 and 9.0)	
3‴	3.30 (*m*)	
4‴	3.37 (*m*)	
5‴$_a$	3.21 (*dd*, *J* = 7.5 and 8.0)	
5‴$_b$	3.46 (*m*)	

*400 MHz, in CD_3OD with TMS as internal reference. The values of the coupling constants are in Hz.

Table 2. ^{13}C NMR spectral data of compounds **4** and **5***

C	5	6
1	131.5	68.7
2	117.1[a]	153.6
3	145.7	127.6
4	144.5	187.3
5	115.3[b]	127.6
6	121.3	153.6
α	72.5	65.6
β	36.5	40.4
1′	104.3	103.7
2′	75.2[c]	74.2[a]
3′	75.8[c]	77.3
4′	72.5	71.0
5′	74.9[c]	74.8[a]
6′	68.5	64.2
1″	127.7	127.2
2″	116.4[a]	116.1
3″	146.4	146.0
4″	149.6	148.8
5″	114.7[b]	114.5[b]
6″	123.0	122.6
α'	116.3[a]	114.9[b]
β'	147.7	146.8
COO	168.5	168.7
1‴	105.2	
2‴	74.8[c]	
3‴	77.5	
4‴	71.1	
5‴	66.8	

*In CD_3OD; TMS as internal reference.

[a–c]These values may be interchanged in the same column.

(rhamnose in 6′) [7] the resonance of the anomeric proton of the rhamnose shows a similar difference.

Calceolarioside D(**6**), $C_{23}H_{26}O_{11}$, showed UV maximum absorptions at 331 and 290 nm and IR bands at 3400 (*br*), 1700 and 1670 cm^{-1}. In respect to calceolarioside B(**2**) the ^{1}H NMR spectrum of **6** (Table 1) presented analogous signals for the *trans*-caffeoyl and the 1,6-disubstituted glucose moieties, whereas the remaining resonances did not agree with the 3,4-dihydroxy-β-phenylethoxy moiety present in the phenylpropanoids so far isolated from *Calceolaria*. Indeed this last pattern of peaks was attributed to a cyclohexa-2,5-dienone structure (δ6.09 and 6.97, two doublets with J = 10 Hz, each accounting for two protons) and a CH_2CH_2O- sequence (Table 1). These assignments, as well as those of the ^{13}C NMR spectrum of **6**, were in good accordance with those of structurally related products, i.e. cornoside (**7**) [8] and hallerone (**8**) [9], accounting for the presence of a (1-hydroxycyclohexa-2,5-dien-4-one)-ethoxy unit linked to the position 1′ of the glucose. Thus the structure of 1′-*O*-β-D-(1-hydroxy-4-oxo-2,5-cyclohexadien)-ethyl-6′-*O*-caffeoylglucopyranoside was assigned to **6**.

The occurrence in plants of different families of phenylpropanoid glycosides and cyclohexanols, as hallerone (**8**) and halleridone (**3**), suggested a common metabolic pathway [9, 10]. This hypothesis is now endorsed by the presence of the cyclohexa-2,5-dienone structure in calceolarioside D.

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were registered on a Bruker AM 400 spectrometer. Separations were performed by CCD with a Craig Post apparatus (200 stages, 10:10 ml, upper and lower phase).

Separation. Acteoside (**4**) (0.2 g, K_r = 0.26), and a mixture of **5** and **6** were obtained from the butanolic residue (1.5 g) by CCD using the solvent system H_2O–AcOEt–*n*-BuOH (10:8:2). The mixture, further purified using H_2O–AcOEt–*n*-BuOH (10:8.5:1.5) gave pure calceolarioside D (0.31 g, K_r = 1.49) and calceolarioside C (0.44 g, K_r = 1.11). Acteoside was identified by direct comparison with an authentic sample.

Calceolarioside C (**5**). Crystals from AcOEt and *n*-hexane, mp 123–125°. $[\alpha]_D^{25} = -2.7°$ (MeOH; *c* 1); UV(MeOH), λ_{max}, nm (log ε): 329, 296, 219 (4.08, 4.00, 4.89); IR (KBr), ν_{max}: 3350, 1690, 1650 and 1060 cm^{-1}. ^{1}H NMR and ^{13}C NMR: Tables 1 and 2, respectively. (Found C, 54.97; H, 5.70; calcd for $C_{28}H_{34}O_{15}$ C, 55.08; H, 5.61%).

Hydrolysis of calceolarioside C (**5**). Compound **5** (100 mg) was treated with 1 N H_2SO_4 (30 ml) at 100° for 1 hr. The reaction mixture was neutralized with $BaCO_3$, the insoluble material was removed by filtration and the soln extracted with AcOEt. In the aq. soln D-glucose and D-xylose were identified by TLC and through the corresponding β-acetyl derivatives separated by

CCD (H_2O–Me_2CO–cyclohexane, 4:6:7) and compared with authentic specimens.

Calceolarioside D (**6**). Colourless amorphous powder. $[\alpha]_D^{25} = -21.5°$ (MeOH; *c* 2); UV (MeOH), λ_{max}, nm (log ε): 331 (4.13), 290 (4.05); IR (KBr), ν_{max}: 3400 (*br*), 2920, 1700, 1670, 1630, 1050 cm^{-1}. ^1H NMR and ^{13}C NMR δ: Tables 1 and 2, respectively. (Found C, 57.59; H, 5.55; calcd for $C_{23}H_{26}O_{11}$, C, 57.74; H, 5.48%).

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PHENYLPROPANOID GLUCOSE ESTERS FROM *PRUNUS BUERGERIANA*

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Key Word Index—*Prunus buergeriana*; Rosaceae; phenylpropanoid glucose esters; caffeic acid esters; *p*-coumaric acid esters; cyanogenic glucoside; mandelonitrile glucoside.

Abstract—Two new phenylpropanoid glucose esters, 6-*O*-caffeoyl-1-*O*-*p*-coumaroyl-β-D-glucopyranose, and 6-*O*-*p*-coumaroyl-D-glucopyranose, along with three known compounds, 1,6-di-*O*-caffeoyl-β-D-glucopyranose, 6-*O*-caffeoyl-D-glucopyranose and (2*R*)-[(6-*O*-caffeoyl)-β-D-glucopyranosyloxy]benzeneacetonitrile were characterized from the bark of the *Prunus buergeriana* using spectroscopic methods.

INTRODUCTION

We have previously reported on the isolation and structural determination of a series of phenylpropanoid glucosides from the bark of *Prunus grayana* Maximowicz [1, 2]. In our continuing chemical examination of phenolic compounds in *Prunus* species, we have now isolated two new phenylpropanoid glucose esters from the bark of *Prunus buergeriana* Miquel. This paper describes the isolation and characterization of these compounds.

RESULTS AND DISCUSSION

A methanolic extract of *P. buergeriana* bark was partitioned with chloroform, and then *n*-butanol. The *n*-butanol soluble part was repeatedly chromatographed over silica gel and Sephadex LH-20 column to give compounds **1**–**5** as amorphous powders.

Compound **1** was analysed for $C_{24}H_{24}O_{12}$ (secondary ion mass spectrometry [SIMS] *m/z* 505 $[M+H]^+$). The ^1H NMR spectrum of **1** showed the existence of two *trans*-olefin systems, aromatic protons of two ABC systems and sugar protons. An anomeric proton signal (δ5.60, *d*, $J = 7.7$ Hz) indicated that the C-1 position of the glucose moiety was acylated. In the ^{13}C NMR spectrum, nine pairs of duplicated signals were observed, which were assigned to the phenylpropanoid moieties. On alkaline methanolysis with methanolic sodium methoxide **1** afforded methyl caffeate and D-glucose. Therefore, caffeic acid was attached to some position of 1-*O*-caffeoyl-β-D-glucopyranose. The location of the residual caffeoyl group was determined to be the C-6 position of the glucose moiety from the chemical shift value in the ^{13}C NMR spectrum of